Recent discovery of widespread *Ixodes affinis* (Acari: Ixodidae) distribution in North Carolina with implications for Lyme disease studies

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ABSTRACT: *Ixodes affinis*, which is similar morphologically to *Ixodes scapularis*, is widely distributed in North Carolina. Collections have documented this species in 32 of 41 coastal plain counties, but no piedmont or mountain counties. This coastal plain distribution is similar to its distribution in Georgia and South Carolina, where it is considered an enzootic vector of *Borrelia burgdorferi sensu stricto*. An updated list of hosts for *I. affinis* in the U.S.A. is included, increasing the number to 15 mammal and one bird species. The presence of questing adults of *I. affinis* from April to November reinforces the need for confirmed identifications of suspected tick vectors of *Borrelia* spirochetes collected during warm months. ***Journal of Vector Ecology* 35 (1): 174-179. 2010.**

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INTRODUCTION

Ixodes affinis Neumann is a Central and South American species belonging to the *Ixodes ricinus* complex, which contains most of the primary vectors of the agents of Lyme borreliosis and a number of other human pathogens (Keirans et al. 1999). This species extends northward into the southeastern U.S.A. (Kohls and Rogers 1953), and previously has been recorded only from Florida, Georgia, and South Carolina (Clark et al. 1998). Oliver et al. (1987) described the immature stages of *I. affinis* and provided distribution, phenology, and host records. *Ixodes affinis* is morphologically very similar to *Ixodes scapularis* Say, the primary vector of Lyme disease borreliae in the eastern U.S.A. (Keirans et al. 1996), which can cause identification problems (Lavender and Oliver 1996). Also, Oliver et al. (2003) reported that in coastal regions of the southeastern U.S.A., *I. affinis* and *Ixodes minor* Neumann are more important than *I. scapularis* in the maintenance of enzootic cycles of Lyme borreliosis spirochetes, including *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt and Brenner, and *Borrelia bissettii* Postic, Ras, Lane, Hendson, and Baranton.

As part of an effort to develop a better understanding of the primary tick vectors and tick-borne pathogens in North Carolina (NC), thousands of ticks were collected between October 23, 2008 and July 31, 2009 by personnel of the Public Health Pest Management (PHPM) Section, NC Department of Environment and Natural Resources, collaborating county personnel, and U.S. Navy personnel. Additionally, preserved ticks collected before October 2008 were re-examined. During this latter process, BAH observed a female collected by WHR in the spring of 2008 that exhibited characters of *Ixodes affinis*, a species not known to be present in NC. In April 2009, this tick was shipped to LAD and confirmed as *Ixodes affinis*. Upon confirmation, the issue of possible misidentifications between October 2008 and April 2009 required the re-examination of the entire collection of *Ixodes* specimens.

This paper presents preliminary results from ongoing investigations of the presence, distribution, and hosts of *I. affinis* in NC. Implications of these findings for future tick-borne disease studies in NC are discussed.

MATERIALS AND METHODS

To date, tick collections in NC have been based on specimens collected on drag cloths or found attached to humans and animals and mailed to PHPM. For this paper the records of *I. affinis* were based on specimens collected through July 31, 2009. Only the first collection of *I. affinis* in each county has been tabulated, regardless of subsequent collections. Location and habitat descriptions were recorded along with GPS coordinates. Special emphasis was placed on descriptions of basic habitats, shade, vegetation height, proximity to water, animal associations, and man-hours of field effort. Specimens were preserved in 95% ethanol for later pathogen assays and identified using the morphological keys in Keirans and Clifford (1978), Keirans and Litwak (1989), and Durden and Keirans (1996). Also, local keys were prepared for the females, males, and nymphs of *Ixodes* ticks confirmed in the state, as well as several other species

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14. ABSTRACT
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likely to occur in NC. The mammal names used here are the scientific names recognized by mammalogists at the North Carolina Museum of Natural Sciences, Raleigh (Benjamin Hess, personal communication).

RESULTS

Following the discovery in NC of the 2008 specimen of *I. affinis*, an intense literature review was conducted, and the first collection record of *I. affinis* in NC was found in Lockhart et al. (1996), who collected this species on deer in 1987 in Hyde County. The specimen(s?) from that collection is no longer available for confirmation (D. E. Stallknecht, personal communication). However, since that publication, neither the NC record nor one for *I. affinis* reported on deer in Arkansas in 1991 by Lockhart et al. (1996) have been acknowledged or cited by tick researchers. Thus, our records of *I. affinis* in NC confirm the previous 1987 record, as do several other confirmed pre-study specimens we found back to 1999. The earliest confirmed NC specimens of *I. affinis* are a pair of mating ticks collected in 1999 in Jones County on a house cat. All *I. affinis* specimens collected to date, except two nymphs, are adults. In 2009 we collected *I. affinis* in January and March through July. Only single specimens were collected during January and March; beginning in April large numbers were collected. Based on collections through July 31, and including all preserved pre-study specimens, 307 *I. affinis* have been confirmed from 32 of 41 coastal plain counties in NC (Table 1). To date, collections of this species have occurred only in coastal plain counties (Figure 1).

Ixodes affinis has been collected on only three hosts in NC: white-tailed deer, dog, and cat. Specimens of this species have also been found crawling on the clothing and skin of collectors, but none have been found attached to humans. Also, two specimens were found on or next to suspended mosquito light traps with CO_2 emitted as an attractant. Currently there are no published records of *I. affinis* parasitizing the white-footed mouse, *Peromyscus leucopus* (Rafinesque), humans, or reptiles, although it may eventually be found attached to these hosts. One bird and 15 mammals are currently recorded as hosts for *I. affinis* in the U.S.A. (Table 2).

DISCUSSION

Following the confirmation of *I. affinis* in NC in April 2009, questions arose concerning how long *I. affinis* has been present in the state. This initiated a re-examination of preserved specimens of *Ixodes* from NC. Specimens collected during the period 1983-1988 were found in the NC State University Insect Museum, Raleigh. That collection period includes the first NC collection of *I. affinis* in 1987 in Hyde County (Lockhart et al. 1996). No specimens of *I. affinis* were found in those collections, but 222 *I. scapularis* were confirmed from Brunswick, Columbus, Currituck, Gates, Hyde, Jones, Martin, New Hanover, Onslow, and Sampson counties, where *I. affinis* does or should occur.

Table 1. North Carolina counties positive for *Ixodes affinis*, including date of first collection, method of collection, and number collected on that date.

County	Date	Method	Number Collected
Beaufort	23-Apr-09	Drag/Flag	28
Bertie	20-May-09	Drag/Flag	3
Bladen	22-Jun-09	Drag/Flag	20
Brunswick	5-Jun-09	Drag/Flag	1
Camden	8-Apr-09	Drag/Flag	1
Carteret	22-Jul-09	Drag/Flag	10
Chowan	10-Mar-09	Drag/Flag	1
Columbus	29-Jul-09	Drag/Flag	1
Craven	3-Jun-09	Drag/Flag	6
Cumberland	28-Jul-09	Drag/Flag	1
Currituck	30-Jul-09	Drag/Flag	1
Duplin	21-Jul-09	Drag/Flag	13
Gates	7-Apr-08	CDC light trap	1
Green	28-May-09	Drag/Flag	1
Halifax	22-May-09	Drag/Flag	1
Harnett	8-Jun-09	Drag/Flag	3
Hyde	Jul-87	Attached Deer	
Jones	19-May-99	Attached Cat	2
Lenoir	8-Jul-09	Drag/ Flag	27
Martin	18-Mar-09	Drag/ Flag	1
Nash	30-Apr-09	Drag/ Flag	2
Northampton	9-Apr-09	Drag /flag	2
Onslow	15-19 Jul 08	Drag/flag	17
Pamlico	9-Mar-09	Drag/Flag	1
Pasquotank	31-Jul-09	On Person	1
Pender	2-Jun-09	Drag/Flag	3
Perquimans	16-Jun-09	Attached Dog	1
Pitt	28-Jul-09	Drag/ Flag	1
Robeson	29-May-09	Drag/Flag	4
Washington	10-Jun-09	Drag/Flag	2
Wayne	5-Jan-09	Drag/Flag	1
Wilson	13-May-09	On Person (crawling)	1

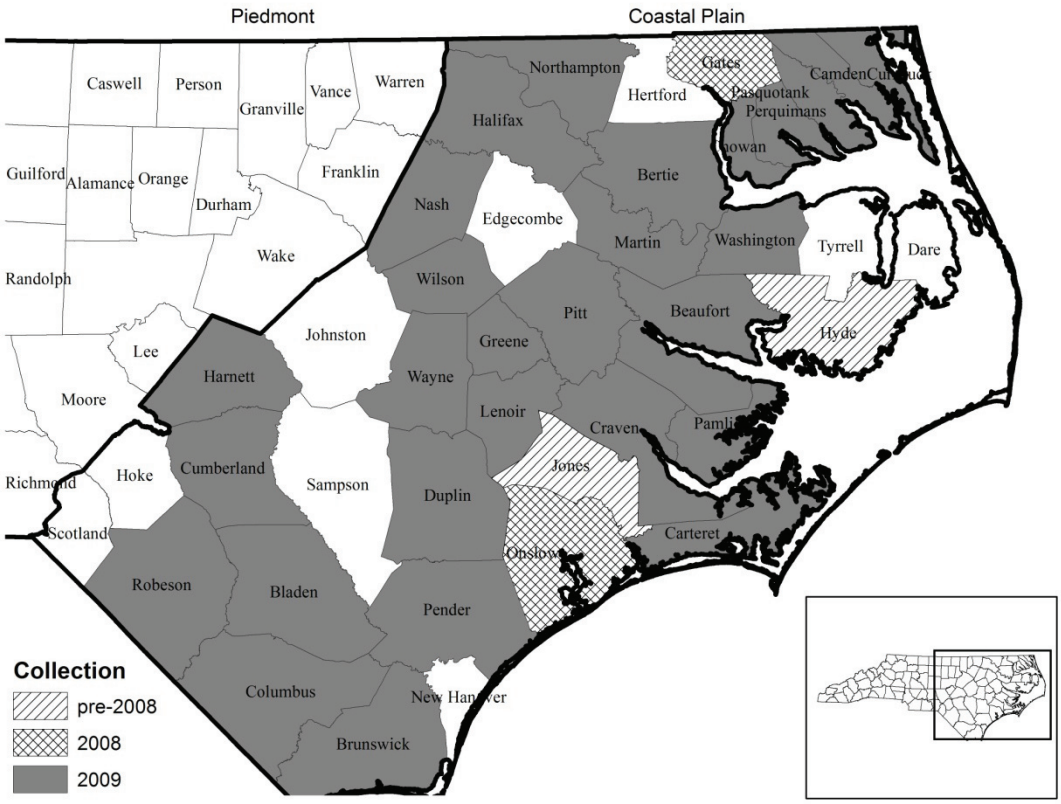

Figure 1. Distribution of *Ixodes affinis* in North Carolina counties as of July 31, 2009, including date of first collection.

Those specimens were collected during the period October-February, all months that coincide with the primary period when *I. scapularis* adults are questing (Goddard 2002). In South Carolina, adults of *I. affinis* normally begin questing in March-April and continue through the summer months (Clark et al. 1998).

During the 1980s and 1990s, a number of tick studies involving *I. scapularis* were conducted in the coastal plain of NC, including, in part, Magnarelli et al. (1986), Levine et al. (1989, 1991), Apperson et al. (1993), Solberg et al. (1995), and Rich et al. (1995). Several of those studies included collection times that overlapped the questing time for adult *I. affinis*, and either used a key (Sonenshine 1979) that did not include *I. affinis*, or did not reference a key or the confirmation of their identifications. Specimens of *I. scapularis* from two other studies were confirmed by Dr. J.E. Keirans. Another study utilized the key of Keirans and Clifford (1978) and confirmed *I. scapularis* specimens from four sites in eastern NC by mitochondrial 16S rDNA sequences. Thus, specimens in some of these studies were collected at times when *I. affinis* would not be questing or on hosts, while the other studies were conducted at times when *I. affinis* could have been collected, if they were present. The absence of *I. affinis* in these published studies and among the 1983-1988 preserved specimens of *I. scapularis* provides preliminary evidence that *I. affinis* may have been uncommon in NC between 1987 and 1999, and probably was transported to NC as larvae or nymphs on migrating birds; alternatively, if established in NC, it may have existed in small separated foci.

Ixodes affinis is now widely distributed in the coastal plain counties of NC (Figure 1), as it is in Georgia and South Carolina (Wells et al. 2004). Also, in certain foci in coastal counties of NC, this species is common. Its distribution extends continuously from South Carolina to the Virginia border, and westward to the edge of the piedmont region. However, despite aggressive collection efforts, no *I. affinis* have been collected in piedmont counties, even those that adjoin positive coastal plain counties. The distribution in NC abuts the southeastern corner of Virginia, which suggests, along with rodent host distributions (Webster et al. 1985), that *I. affinis* may also occur in Virginia.

In Georgia and South Carolina, abundance of *I. affinis* has been associated with the distributions of three rodent species - cotton mouse, hispid cotton rat, and eastern wood rat (Durden and Oliver 1999, Clark et al. 2001, Oliver et al. 2003) - with the first being the most important host for this species. These rodents serve as the primary hosts for the immature stages of *I. affinis* (Clarke et al. 1998), and they are also major amplifying hosts for *Borrelia burgdorferi s. s.* and *Borrelia bissettii.* Furthermore, *I. affinis* serves as a primary enzootic vector for *Borrelia burgdorferi s. s.* in the coastal plains of those two states (Oliver et al. 2003). In NC, the distribution of *I. affinis* overlaps the distributions of these three rodent species, except for the eastern wood rat, which is confined to the southeastern part of the coastal plain and the mountains. The distribution of *I. affinis* also overlaps those of the marsh rice rat along the coast, and the white-footed mouse in northern portions of the coastal plain (Webster et al. 1985).

Table 2. Chronological list of mammal and bird hosts for *I. affinis* in the United States.

Common name	Scientific name	Primary Reference
Bobcat	*Lynx rufus* (Schreber)	Kohls and Rogers (1953)
Dog	*Canis lupus familiaris* L.	"
White-tailed deer	*Odocoileus virginianus* Zimmermann	Kellogg et al. (1971)
Cougar	*Puma (Felis) concolor* (L.)	Forrester et al. (1985)
Carolina wren	*Thyrothorus ludovicianus* (Lathum)	Oliver et al. (1987)
Cotton mouse	*Peromyscus gossypinus* (Le Conte)	"
Cotton rat	*Sigmodon hispidus* Say and Ord	"
Eastern wood rat	*Neotoma floridana* (Ord)	"
Gray squirrel	*Sciurus carolinensis* Gmelin	"
Southern short-tailed shrew	*Blarina carolinensis* (Bachman)	"
Virginia opossum	*Didelphis virginiana* (Kerr)	"
Raccoon	*Procyon lotor* (L.)	"
Marsh rice rat	*Oryzomys palustris* Harlan	Durden and Oliver (1999)
Cottontail rabbit	*Sylvilagus floridanus* (Allen)	Nelder and Reeves (2005)
American black bear	*Ursus americanus* (Pallas)	Yabsley et al. (2009)
Housecat	*Felis catus* (L.)	This report

Spielman et al. (1985) implicated the white-footed mouse as the primary amplifying mammal host of *Borrelia burgdorferi s. s.* in the northeastern Lyme disease cycle, yet Webster et al. (1985) indicated that the white-footed mouse does not occur in coastal counties of South Carolina or NC south of Pamlico Sound. If this distribution has not changed it raises the question: Is *I. affinis* a primary enzootic vector of *B. burgdorferi s. s.* in the rodent cycle in southeastern NC?

As an indication of both the importance of accurate tick identification and the potential significance of *I. affinis* in the transmission cycle of *B. burgdorferi s. s.* in eastern NC, 17 *Ixodes* adults collected on drags in Onslow County in July 2008 were initially identified as *I. scapularis*, but when re-examined due to unusual seasonal occurrence, all 17 were actually found to be *I. affinis*. Six (35%) of those ticks tested positive for *B. burgdorferi s. s.* (BFP, unpublished), yet this species has never been documented to bite humans. This positive rate is slightly higher than the positive rate found in *I. affinis* in South Carolina, where this tick is a primary enzootic vector of *B. burgdorferi s. s.* in rodent hosts (Clark et al. 2002).

North Carolina has a greater diversity of rodents and reptiles with differing distributions, a more diverse tick fauna utilizing these hosts, and more diverse *Borrelia* populations (Ryan et al. 1998, 2000) than those found in the northeastern U.S.A. This suggests that there may be several different basic cycles of *Borrelia burgdorferi s. s.* in the state, each operating in different regions, as in the diverse *Borrelia* life cycles known from Georgia and South Carolina (Oliver et al. 2003). Thus, investigations of these spirochetes and their tick vectors in NC should initially confirm the identifications of ticks, rodents, and/or reptiles in targeted study sites and not rely on information from the *Borrelia* life cycle prevalent in the northeastern U.S.A. This will provide valuable information about: seasonality of different life stages of each tick species, selection of appropriate collection methods, rodent and/or reptile species to examine for tick immature stages, medium to large mammal hosts on which adult ticks will occur, and likely amplifying rodent hosts for *Borrelia* spirochetes.

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